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# PEGylated dendritic nanoarchitechture improves mean survival time of $BDF_1$ mice bearing myelogenous k -562 leukemia

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ABSTRACT

**Objective:** To developing and exploring the use of PEGylated poly (propylene imine) dendritic architecture for the delivery of an anti leukemic activity of Prednisolone. **Methods:** For this study, PEGylated poly (propylene imine) dendritic architecture was synthesized and loaded with Prednisolone and targeted to the ascetic form of myelogenous leukemia k-562 cellines in hybrid mice BDF1, was used as tumor model. The antileukemic activity was assessed by use of the criterion T/C %, where T was the mean survival time (MST, days) of the drug treated mice, bearing k-562 leukemia and C - the mean survival time (MST, days) of untreated control animals, bearing the same leukemia cellines. **Results:** An antileukemic activity of the studied Prednisolone loaded PEGylated Polypropyleneimine (PPI) dendrimer was found to have increasing the mean survival time of the k-562 myelogenous leukemia cellines bearing BDF1 mice. The criterion "increase of life span" (ILS%) reached maximally 270.1% for the drug loaded dendrimer. **Conclusion:** The studied dendrimer with Prednisolone showed lower toxicity with improved antileukemic activity in comparison with free Prednisolone. The further experiments in this field are in progress, aiming to design better dendritic formulations, with potential clinical use

## 1. Introduction

Dendrimer represents a novel type of polymeric material. It is also known as starburst[1] or cascade[2] or molecular trees[3] or arborols, or polymers. They attract the increasing attention of all because of their unique structure, high degree of control over molecular weight and the shape that has led to the synthesis of unimolecular micelles[4-7]. Considering the use of dendrimers for drug delivery, it is necessary that they are nontoxic and biocompatible. However, it has been demonstrated that widely used dendrimers, such as PAMAM and poly (propyleneimine) (PPI) dendrimers bearing primary amino group termini, are quite cytotoxic, and also these dendrimers were cleared rapidly from the circulation when administered intravenously[8]. Poly ethylene glycol (PEG) is typically a clear, colorless odorless substance that is soluble in water, stable to heat, inert to many chemical agents, that does not hydrolyze or deteriorate, and is generally non-toxic, PEG is considered to be biocompatible, which is to say that PEG is capable of coexistence with living tissue or organisms without causing harm, as reviewed earlier[9]. It has been shown that covalent attachment of poly (ethylene glycol) to proteins decreases their immunogenicity and increases their circulation time. Moreover, a number of studies have demonstrated that poly (ethylene glycol) chains grafted to surface of polymer micelles and liposomes suppress their interaction with plasma proteins and cells and prolong their blood elimination half-life. On the basis of these findings, it seems that dendrimers covered with poly (ethylene glycol) grafts are attractive compounds as drug carriers in in vivo. The purpose of the present experimental investigations was to assess the antileukemic activity of an Prednisolone loaded PEGylated Polypropyleneimine (PPI) dendrimer in comparison with free Prednisolone.

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## 2. Materials and methods

### 2.1. Materials

PEG4000, Reney Nickel (Sigma, Germany), Raney Nickel (Merck, India), Triethylamine, Ethylenediamine, Acrylonitrile (CDH, India), N, N dicyclohexyl carbodiimide (DCC), Cellulose dialysis bag (MWCO 12-14 Kda, Himedia, India), Prednisolone was a benevolent gift from Shasun Pharmaceuticals, Chennai, India.

### 2.2. Methods

#### 2.2.1. Synthesis of 5.0G PPI dendrimer

5.0G PPI dendrimer was synthesized by following the procedure reported by (De Brabender-Van Den Berg and Meijer) using EDA as initiator core. Briefly, ethylenediamine (EDA) was used as initiator core and acrylonitrile was added to it in a double Michael addition-reaction method to produce half generation (-CN terminated), followed by heterogeneous hydrogenation using Reney Nickel as catalyst to produce full generation (-NH2) dendrimers. The reaction sequence was repeated cyclically to produce PPI dendrimers up to fifth generation (PPI-5.0G) as shown in Figure 1.

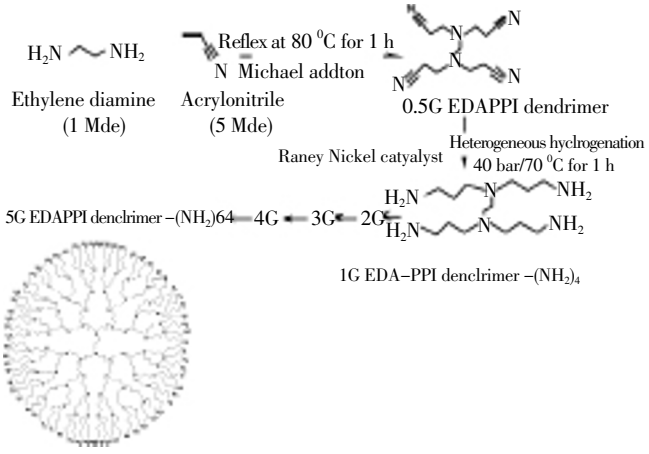
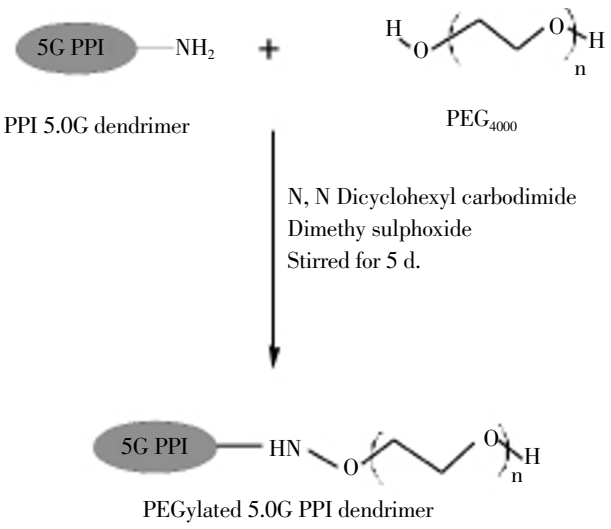



**Figure 1.** Schematic diagram for synthesis of PPI-5G dendrimer.

#### 2.2.2. Synthesis of PEGylated 5.0G PPI Dendrimers

To a solution of 5G EDA-PPI dendrimer (0.01 mmol) in dimethyl sulfoxide (DMSO) (10 mL), PEG 2000 (0.32 mmol) in DMSO (10 mL) and N, N dicyclohexyl carbodiimide (DCC) (0.32 mmol) in DMSO (10 mL) were added and the solution was stirred for 5 d at room temperature. The product was precipitated by addition of water, filtered and dialyzed (MWCO 12-14 Kda, Himedia, India) against double distilled water for 24 h to remove free PEG 2000, DCC and partially PEGylated dendrimers followed by lyophilization (Heto drywinner, Germany).The preparation of PEGylated 5.0 G PPI dendrimers was shown in Figure 2.

### 2.3. Drug loading in formulation




The known molar concentrations of EDA-PPI dendrimer and PEGylated 5.0G dendrimers were dissolved separately in methanol and mixed with methanolic solution of Prednisolone (100 mol). The mixed solutions were incubated with slow magnetic stirring (50 rpm) using teflon beads for 24 h. These solutions were twice dialyzed in cellulose dialysis bag (MWCO 1000 Da Sigma, Germany) against double distilled water under sink conditions for 10 min to remove free drug from the formulations, which was then estimated spectrophotometrically ($\lambda$max 248 nm) (UV-1601, Shimadzu, Japan) to determine indirectly the amount of drug loaded within the system. The dialyzed formulations were lyophilized and used for further characterization.

### 2.4. Antileukemic activity

The in vivo studies were performed in male hybrid BDF1 mice. The antileukemic activity was studied on ascitic form of myelogenous k-562 leukemia, with transplantation dose of $1\times10^5$ tumor cells/mouse, on day 0, intraperitoneally (i.p.). Prednisolone and Prednisolone loaded PEGylated Polypropyleneimine (PPI) dendrimer were introduced intraperitoneally, once a day, on day 1, day 4 and day 8 after the tumor transplant. The antileukemic activity was assessed by use of the criterion T/C %, where T was the mean survival time (MST, days) of the drug treated mice, bearing k-562 leukemia and C - the mean survival time (MST, days) of untreated control animals, bearing the same leukemia cellines[10].

### 2.5. Statistical analysis

The activity was assessed by use of the criterion T/C%, where T was the mean survival time (MST, days) of the drug treated mice, bearing k-562 myelogenous leukemia

and C – the mean survival time (MST, days) of untreated control animals, bearing the same leukemia. T/C%> 125% is considered as significant.

## 3. Result

The polypropyleneimine dendrimer was synthesized by using ethylenediamine as a core. The synthesized dendrimer were further PEGylated with PEG4000. The PEGylated dendrimer is used as carrier system, in which Prednisolone was loaded and drug entrapment efficiency was calculated as (81.2±0.03). The antileukemic activity was assessed by use of the criterion T/C%. The results obtained from the effect of Prednisolone and its Prednisolone loaded PEGylated Polypropyleneimine (PPI) dendrimer on BDF1 hybrid mice-bearing K-562 leukemia are shown on the Table 1. According to these results, the free Prednisolone exhibited a pronounced and dose-related antileukemic activity on mice-bearing K-562 leukemia. An increase of the free Prednisolone dose over 0.25 mg/kg ×3, i. p., caused an increase in its acute toxicity. This fact was registered by the progressive decrease in the ratio T/C (treated/control). The dose of the free Prednisolone of 1.5 mg/kg×3, i. p., was toxic (T/C% < 125%). The Prednisolone loaded PEGylated Polypropyleneimine (PPI) dendrimer exhibited an antileukemic activity against acute lymphocytic K-562 in BDF1 mice, in four of the used doses from 0.5 mg/kg× 3 to 8.0 mg/kg×3, i. p., with T/C% varying between 195.1% and 270.1%. The experimental results on activity of the Prednisolone loaded PEGylated Polypropyleneimine (PPI) dendrimer showed that an increase in dose levels of equivalent to the free drug led to an increase in the ratio T/C, indicating lower toxicity. The dose of 8.0 mg/kg×3, i. p., was not toxic (T/C% = 270.1%).

**Table 1.**
Antileukemic activity of free prednisolone and prednisolone loaded PEGylated PPI dendrimer on BDF1 hybrid mice-bearing K-563 leukemia.

| Drug and formulation | Dose (mg/kg) ×3, i.p | MST ( in days) | T/C (%) |
|---|---|---|---|
| Prednisolone | 0.25 | 19.4 | 179.6 |
| | 0.5 | 17.3 | 166.3 |
| | 1.0 | 14.8 | 142.3 |
| | 1.5 | 12.5 | 120.1 |
| | 0.5 | 20.3 | 195.1 |
| Prednisolone loaded | 1.0 | 21.6 | 207.6 |
| PEGylated PPI | 2.0 | 23.2 | 223.0 |
| dendrimer | 4.0 | 26.7 | 256.7 |
| | 8.0 | 28.1 | 270.1 |
| Untreated control | 0 | 10.8 | |

MST – mean survival time (days); T – survival time of treated mice (days); C – survival time of control mice (days); Significant antileukemic effect at T/C% > 125% was accepted. *Toxic dose at T/C% < 125%.

## 4. Discussion

The antileukemic activity of the Prednisolone loaded PEGylated dendrimers are shown more significant improvement in mean survival time of cancer affected mice than the free Prednisolone, that was favorable by clinical point of view. The chemical and pharmacological investigations in this field are in progress, aiming to analyse the results and trying to design better formulation of selected antitumor drugs with dendrimers, for potential clinical use.

## Conflict of interest statement

The authors report no conflict of interest.

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